Field Crops Research 221 (2018) 182–195

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Interactive effects of high temperature and drought stress during stem elongation, anthesis and early grain filling on the yield formation and photosynthesis of winter wheat

Marcela Hlaváčová[a,b], Karel Klem[a,b], Barbora Rapantová[a,c], Kateřina Novotná[a,b], Otmar Urban[a], Petr Hlavinka[a,b], Pavlína Smutná[b], Vladimíra Horáková[d], Petr Škarpa[b], Eva Pohanková[a,b], Markéta Wimmerová[a,b], Matěj Orság[a,b], František Jurečka[a,b], Miroslav Trnka[a,b,*]

[a] Global Change Research Institute CAS, Bělidla 986/4a, 603 00 Brno, Czech Republic
[b] Mendel University in Brno, Faculty of Agronomy, Zemědělská 1, 613 00 Brno, Czech Republic
[c] Mendel University in Brno, Faculty of Forestry and Wood Technology, Zemědělská 3, 613 00 Brno, Czech Republic
[d] Central Institute for Supervising and Testing in Agriculture, Hroznová 65/2, 656 06 Brno, Czech Republic

ARTICLE INFO

Keywords:
Drought stress
Heat stress
Photosynthesis
Triticum aestivum
Yield formation

ABSTRACT

Heat waves and drought periods are expected to become more frequent due to climate change. This may cause a critical decline in future crop yields. However, insufficient knowledge of the interactive effects of high temperature and drought stress at specific growth stages is the cause of numerous uncertainties in modeling impacts of climate change on field crop growth and yield. Hence, the aim of this study was to investigate the effects of interactions between the short-term exposure (3 and 7 days) of two winter wheat genotypes to elevated temperature and drought stress on yield formation and photosynthetic parameters. Winter wheat plants grown under ambient conditions were subjected to four temperature regimes (with maxima at 26, 32, 35 and 38 °C) and drought in growth chambers at three critical growth stages (beginning of stem elongation – DC 31, beginning of anthesis – DC 61, and medium milk ripe – DC 75). The response of yield formation parameters was obviously modulated by variety and growth stage. Grain number was more affected by drought at DC 31 and by the temperature at DC 61. Grain weight per spike was reduced by drought stress similarly at all growth stages, but the results indicated the increasing sensitivity of this parameter to a temperature at the later growth stage. Although yield parameters only changed slightly with the length of heat and drought stress, the photosynthetic parameters were strongly affected, particularly by longer drought and the interactive effect of high temperature and drought stress. Higher temperature significantly increased the negative impact of drought on CO_2 assimilation rate. Photosynthetic parameters were less affected by combined high temperatures and drought stress at DC 61 as compared to other growth stages investigated. The larger effect at the later growth stage (DC 75) can be attributed to induced senescence, among other factors, particularly in the Bohemia variety. The Tobak variety appears to be more tolerant to combined high temperatures and drought stress in terms of photosynthetic parameters. Based on the relationships between heat degree-days (HDD) and grain weight per spike we demonstrated the potential of HDD to integrate length and intensity of heat stress at different growth stages, particularly for estimation effects on yield parameters. It can be summarized that although the short-term combination of high temperatures and drought causes significant reductions in photosynthetic parameters, the impact on yield formation is much lower, indicating fast recovery of photosynthetic processes and compensation in yield formation parameters.

* Corresponding author at: Global Change Research Institute CAS, Bělidla 986/4a, 603 00 Brno, Czech Republic.
E-mail addresses: hlavacova.m@czechglobe.cz (M. Hlaváčová), klem.k@czechglobe.cz (K. Klem), rapantova.b@czechglobe.cz (B. Rapantová), novotna.k@czechglobe.cz (K. Novotná), urban.o@czechglobe.cz (O. Urban), petr.hlavinka@mendelu.cz (P. Hlavinka), pavlina.smutna@mendelu.cz (P. Smutná), vladimira.horakova@ukzuz.cz (V. Horáková), petr.skarpa@mendelu.cz (P. Škarpa), eva.pohankova@mendelu.cz (E. Pohanková), wimmerova.m@czechglobe.cz (M. Wimmerová), matej.orsag@mendelu.cz (M. Orság), jurecka.f@czechglobe.cz (F. Jurečka), miroslav.trnka@mendelu.cz (M. Trnka).

https://doi.org/10.1016/j.fcr.2018.02.022
Received 3 July 2017; Received in revised form 19 February 2018; Accepted 21 February 2018
Available online 23 March 2018
0378-4290/ © 2018 Published by Elsevier B.V.

1. Introduction

Drought as well as heat stress (high temperature stress) represent the major abiotic stress factors affecting growth, development and production of plants (Noohi et al., 2009; Hasanuzzaman et al., 2012; Hossain et al., 2012; Bilal et al., 2015; Lobell et al., 2015). Moreover, these stresses often occur at the same time (Rizhsky et al., 2004) and are physically intertwined at least in some regions (Hirschi et al., 2011).

The annual global temperature is predicted to increase by 1.8–4.0 °C until the end of the 21st century (Bita and Gerats, 2013). The increase in occurrences of extreme weather events (Zheng et al., 2012) such as short periods of very high temperatures (> 33 °C) (Wardlaw et al., 1989; Stone and Nicolas, 1994) and drought spells (Li et al., 2009) is also expected under future climate scenarios (e.g., Trnka et al., 2011), and it represents a significant risk for crop production (Wardlaw et al., 1989; Stone and Nicolas, 1994; Zheng et al., 2012). Wheat, as the globally second most widely grown cereal crop (Reyer et al., 2013), is very susceptible to drought stress, particularly at the developmental stages of anthesis and grain-filling (Trnka et al., 2014). Moreover, the susceptibility is likely to increase in future climate conditions across large portions of European wheat-producing regions (Trnka et al., 2015) including the Czech Republic (Trnka et al., 2016a). The damage temperature threshold for wheat is estimated to be 31 °C during the flowering stage and 35 °C during the grain filling stage (Niwas and Khichar, 2016).

Both heat and drought stress directly or indirectly regulate a number of biochemical and physiological processes. In addition to directly affecting plant growth (Skirycz and Inzé, 2010), the most affected processes are related to photosynthesis (Chaves et al., 2009; Sharkey, 2005). The direct effect on photosynthesis is mediated by stomatal closure or mesophyll conductance (Flexas et al., 2007) and occurs much earlier, while the indirect effect is due to downregulation of photosynthetic metabolism occurring under prolonged or more severe stress (Lawlor and Cornic, 2002; Salvucci and Crafts-Brandner, 2004). Drought and/or heat stress reduce membrane integrity and increase lipid peroxidation, leading to a reduction of chlorophyll content (Bolhar-Nordenkampf et al., 1991; Mafakheri et al., 2010) and functionality of leaves (Al-Khatib and Paulsen, 1984) and stress-induced premature senescence (Silva et al., 2010). Heat stress thus accelerates not only whole plant senescence but also the grain filling process, leading to an earlier maturation and a shorter reproductive phase (Farooq et al., 2011), which result in a significant yield loss (Barnabas et al., 2008). The leaf senescence in wheat was found to be accelerated at temperatures above 31 °C shortly after anthesis and during grain filling (Asseng et al., 2011).

The responses of plants to drought and heat stress are often species-specific, but there are also differences among varieties within a species (Ristic and Cass, 1992; Demirevska et al., 2008; Vassileva et al., 2011). It is evident that the evaluation of photosynthetic responses and changes in chlorophyll content may provide relevant selection criteria for breeding new genotypes that are tolerant to higher temperatures and drought stress (Mohammadi et al., 2009; Monneveux et al., 2006; Camejo et al., 2005).

Field crops are known to be more prone to heat stress in reproductive developmental stages compared to vegetative phases (Vara Prasad et al., 2017); in addition, the response of each yield component of wheat depends on the specific crop and the developmental stage in which the stress occurs (Stone and Nicolas, 1995; Ferris et al., 1998; Calderini et al., 1999; Gibson and Paulsen, 1999). Nevertheless, the information on responses of various field crops to heat stress during reproductive stages (especially anthesis) at a finer scale of exposure to this stress is still less known (Vara Prasad et al., 2017). Moreover, studies about the responses of yield components of various wheat varieties to heat stress at different developmental stages are still rare (Kumar et al., 2016). While a retrospective approach based on regional yield statistics across Europe does not allow one to disentangle individual influences completely (e.g., Trnka et al., 2016b), there seems to be a shift towards an increasing sensitivity of wheat to heat and drought stress over the past 120 years, as Trnka et al. (2012) suggested.

Complete knowledge of the responses of wheat varieties to the most serious abiotic factors such as drought or heat stress and their interactions is necessary for ensuring sustainable crop production under the future climate conditions. Therefore, the aim of this study was to assess how short-term exposure (3 and 7 days) to heat and drought stress and their combination, as a simulation of heat waves that are expected to be more frequent during vegetation seasons under future climate conditions, affect the photosynthetic parameters and yield formation components (grain number, spike productivity) at different growth stages. The main hypotheses tested were as follows: (1) Under short-term exposure to drought and heat stress, the photosynthetic recovery and compensation between yield formation parameters result in a non-proportional reduction of individual yield parameters. (2) Wheat genotypes differing in yield formation have different sensitivities to heat and drought stress at individual critical growth stages for yield formation. (3) The sensitivities of yield formation parameters to heat and drought stress and their interaction change with growth stage.

2. Material and methods

2.1. Plant material

Two of the most widely grown winter wheat varieties (*Triticum aestivum* L.) in the Czech Republic were sown (2 seeds per pot) in plastic pots (10.5 × 10.5 × 21 cm). The plants were cultivated under ambient weather conditions in a vegetation hall at the Mendel University in Brno (235 m a.s.l.; 16°36′48.64716“N, 49°12′36.62892“E). The soil used for cultivation was a topsoil (0–30 cm) from the Polkovice experimental station (199 m a.s.l.). The soil is a Luvic Chernozem with a silt-clay texture (26% clay, 68% loam, 6% sand); pH_{CaCl_2} 7.4; C_{tot} 2.66%; N_{tot} 0.25%; and the contents of P, Ca, Mg and K (Mehlich III) were 143, 6045, 252 and 567 mg kg^{-1}, respectively. The floor of the vegetation hall was concrete and the hall was covered with a wire netting roof to enable exposure of the pots to the ambient weather conditions. The plants in the vegetation hall were (1) irrigated (in total, 80 mm per month in the vegetation hall) to prevent the plants from drought stress and (2) protected against pests and diseases using recommended plant protection products and fertilized (see Table A in Supplementary materials) during their placement in the vegetation hall.

2.2. Environmental conditions during temperature and drought stress treatment in growth chambers

The responses of the wheat plants to heat and drought stress were assessed under fully controlled environmental conditions in growth chambers at three developmental stages: beginning of stem elongation (DC 31), beginning of anthesis (DC 61) and the early grain filling period (DC 75) at two exposure lengths (three and seven days). The protocols used in the growth chambers provided a daily schedule of air temperature (T), photosynthetically active radiation (PAR), and relative humidity (RH) close to the natural conditions. The temperature regimes differed in temperature maxima (T_{max}): 26, 32, 35 and 38 °C. The RH and PAR protocols were the same in each treatment (Table 1). Drought stress was established by splitting the pots into two subtreatments within each temperature treatment: (1) well-watered (*control*), where the soil volumetric moisture was maintained at approximately 30% and (2) drought-stressed, where the volumetric soil moisture was maintained at approximately 15–20%. Each combination of temperature and drought treatment was replicated in 7 pots, and the replications were randomized in space and time. After the heat and temperature treatment at each growth stage, the plants were moved back to the vegetation hall and regularly irrigated. The plants grew in the vegetation hall up to the full ripening stage (DC 92), when the manual harvest was

Table 1
Daily schedule of environmental conditions in the growth chambers. Environmental conditions changed continuously between two time points. T – air temperature, T_{max} – temperature maxima (26, 32, 35 and 38 °C for individual temperature treatments), PAR – photosynthetically active radiation, RH – relative air humidity.

Time	T (°C)	PAR (μmol $m^{-2} s^{-1}$)	RH (%)
0:00–4:00	20–18	0	85–90
4:00–6:00	18	0	90
6:00–12:00	18–T_{max}	0–1500	90–45
12:00–14:00	T_{max}	1500	45
14:00–20:00	T_{max}–22	1500–0	45–75
20:00–24:00	22–20	0	75–85

Table 2
Heat degree-days (HDD) above 30 °C calculated according to Liu et al. (2016) for wheat plants exposed to the stress regimes for 3 and 7 days (an interpolation of temperature course between time steps inside the growth chambers was calculated).

Chamber	HDD	
T_{max} (°C)	3 days	7 days
26	0	0
32	0.79	1.85
35	2.58	6.03
38	4.75	11.08

carried out (July 2016). The total heat stress inside the growth chambers, expressed as heat degree-days (with a temperature threshold of 30 °C) calculated according to Liu et al. (2016), are presented in Table 2.

2.3. Measurements

The volumetric soil moisture was controlled with an ML3 ThetaProbe (Delta-T Devices, Cambridge, United Kingdom) sensor (see Table B in Supplementary materials). The rate of leaf senescence, i.e., leaf chlorophyll content, was measured *in vivo* using a polyphenol and chlorophyll-meter Dualex 4 Flav (Force A, Orsay, France) after 3 and 7 days of exposition to the stress conditions. An open gas-exchange system Li-6400 XT (Li-Cor Biosciences, Lincoln, NE, USA) was used to estimate the light saturated (1500 μmol photons $m^{-2} s^{-1}$) CO_2 assimilation rate (A_{max}) and stomatal conductance for H_2O (G_{Smax}). All physiological measurements were done at the temperature maxima of individual treatment between 10:00 and 15:00 (CET). Yield formation parameters such as grain number (GN) and grain weight (dry mass) per spike (GW) were determined after manual harvest and drying of grains at 70 °C for 48 h. Thousand grain weight (TGW) was calculated from measured GW and GN.

2.4. Statistical analysis

The least square means (means within each replication/pot) for the yield formation parameters and chlorophyll index measurements were calculated before statistical analyses. Then, the data were tested for normality using the Kolmogorov-Smirnov test and homogeneity of variance (Levene's test). The two-way ANOVA followed by Tukey's post hoc test was performed within each genotype, growth stage and length of treatment using Statistica 12.0 software (StatSoft, Tulsa, USA). The graphs were created in SigmaPlot software (Systat Software, San Jose, USA).

3. Results

3.1. Grain number per main spike

GN decreased with the temperature and drought treatment at later growth stages, particularly in the Tobak variety (Fig. 1). The ANOVA (Table 3) revealed significant effects of drought stress on GN at DC 31 in both varieties and at DC 61 in the Tobak variety. In general, the effect of drought decreased at later growth stages. Increasing temperature had slightly positive effects on GN at DC 31, particularly in the well-watered control, while negative effects were found at later stages. However, the effect of temperature was significant in the Tobak variety only (Table 3). In the Tobak variety, it was evident that there was a decrease in GN at the maximum temperature of 38 °C, specifically at DC 61. A gradual decrease in GN with temperature was then observed in the Tobak variety at DC 75, namely, under drought stress. The length of treatment changed the responses only slightly. Although generally no significant interactions between effects of drought stress and temperature were found (Table 3), the drought effect increased with higher temperature in the Bohemia variety at growth stage DC 31 and partly at DC 75.

3.2. Grain weight per main spike and thousand grain weight

GW, an integrating parameter of GN and TGW, was significantly affected by both temperature and drought stress in both varieties and at all growth stages (Table 3). On the other hand, thousand grain weight (data not shown) was mainly affected at DC 31 and DC 75. At DC 75, the effects of both factors on TGW were higher in the Tobak variety. Significant interactions between temperature and drought stress effects on GW were found in the Bohemia variety at DC 31 (7 days) and DC 61 (3 days) and in the Tobak variety at DC 61 (3 days). Significant interactions were also found for the parameter of TGW at DC 31 (both lengths of treatment) in the Bohemia variety, and DC 31 (7 days) or DC 75 (7 days) in the Tobak variety. The effect of drought stress on GW was highest in the Bohemia variety at DC 31 and decreased with later growth stages, while in the Tobak variety, it was similar across all growth stages (Fig. 2). In the Tobak variety, it was also evident that there was a consistent negative effect of increasing temperature. This effect, at DC 31 and DC 75, was characterized by a gradual decline, while at DC 61, the negative effect on GW was characterized by a more pronounced decrease in the highest temperature (38 °C). On the other hand, GW in the Bohemia variety showed positive effects to increasing temperatures at DC 31 and negative effects at DC 61 and DC 75. In the Tobak variety at DC 61, increasing temperatures strengthened the negative impact of drought, resulting in significant differences between plants under drought stress and the control at temperatures 32 °C and higher. In contrast, at the same growth stage (DC 61) in the Bohemia variety, the drought effect decreased with increasing temperatures. The effect of temperature and drought stress on TGW was similar to the effect on GW, however, the differences were smaller and also less significant. Generally, drought stress slightly reduced the TGW. A decrease in TGW caused by high temperature (35–38 °C) was also small and was observed for the variety Tobak at DC 31 and for both varieties at DC 75. A decrease in the TGW was evident only in interaction with the drought stress and only for the variety Tobak at DC 61. The statistically significant effect of temperature was observed only in variety Tobak, particularly at DC 31. The effect of drought stress on TGW was found only at temperature 26 °C, in variety Bohemia at DC 31 and in variety Tobak at DC 75 (Fig. 3).

3.3. Chlorophyll index

The results of the *in vivo* determination of chlorophyll content (chlorophyll index; Fig. 4) showed that the effects of temperature and drought stress were small and in most cases statistically insignificant, particularly at an early growth stage (DC 31). The effect of drought increased in variety Tobak at DC 61 and in variety Bohemia at DC 75. However, this negative effect of drought stress on chlorophyll index was manifested only at temperatures in the range of 32–35 °C, while at lower and higher temperatures the differences between control and

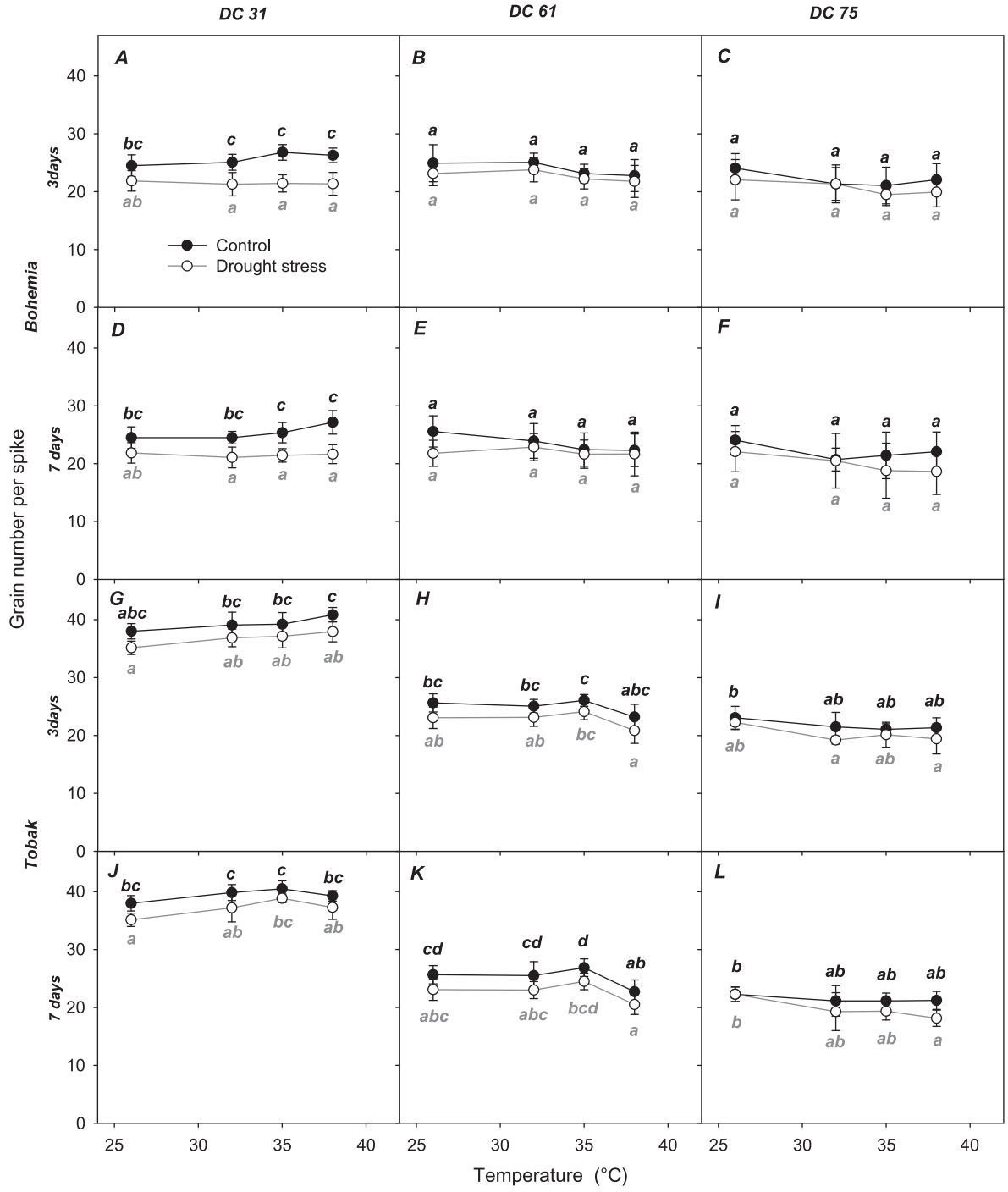

Fig. 1. Grain number per main spike in winter wheat varieties Bohemia (A–F) and Tobak (G–L) under exposure to temperature and drought stress treatments for 3 (A–C, G–I) and 7 (D–F, J–L) days at growth stages DC 31–beginning of stem elongation (A, D, G, J), DC 61–beginning of anthesis (B, E, H, K) and DC 75–medium milk ripe (C, F, I, L). Means (points) and standard deviations (error bars) are presented (n = 7). Different letters denote statistically significant differences among means tested by ANOVA with Tukey's post hoc test (P = 0.05) within each variety, growth stage and length of treatment separately.

drought-stressed plants were insignificant. At these temperatures, generally, chlorophyll index also slightly increased as compared to 26 °C. This increase was more pronounced in variety Bohemia, particularly after 7-day treatment. The observed decline in chlorophyll index at 38 °C as compared to 35 °C was negligible and statistically insignificant in both genotypes irrespective of water availability and growth stage. The more sensitive variety to drought in terms of chlorophyll content (Bohemia) also showed at growth stage DC 75 that the maximum of temperature response was shifted under drought stress towards higher temperatures (38 °C). In general, however, the effect of either high temperature or drought stress on chlorophyll content may be considered to be relatively low for both lengths of treatment (3 and 7 days).

3.4. Gas exchange parameters

Both light saturated CO_2 assimilation (A_{max}) and stomatal conductance (G_{Smax}) showed significant responses to temperature and drought stress at all growth stages, and in several cases, there were also significant interactions (Table 3). A_{max} also exhibited a typical shift of

Table 3
Significance (P-values) of temperature (T) and drought (D) effects and their interactions (T × D) on an individual yield and physiological parameters tested by two-way ANOVA. A_{max} represents a light saturated CO_2 assimilation rate, G_{Smax} is a light saturated stomatal conductance for H_2O.

		Bohemia						Tobak					
		DC 31		DC 61		DC 75		DC 31		DC 61		DC 75	
		3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Grain number per spike	T	0.353	0.083	0.109	0.130	0.079	0.140	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.004**	**0.004**
	D	**< 0.001**	**< 0.001**	**0.029**	0.099	0.067	**0.033**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.004**	**0.002**
	T x D	0.144	0.154	0.958	0.741	0.742	0.640	0.873	0.697	0.953	0.899	0.647	0.220
Grain weight per spike	T	**< 0.001**	**< 0.001**	**< 0.001**	**0.017**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	D	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	T x D	0.085	**0.005**	**0.040**	0.259	0.501	0.595	0.745	0.063	**0.019**	0.435	0.720	0.906
Thousand grain weight	T	**0.005**	**< 0.001**	0.360	0.920	0.117	**0.001**	**< 0.001**	**< 0.001**	0.137	**< 0.001**	**0.028**	**< 0.001**
	D	**< 0.001**	**< 0.001**	0.097	0.287	**0.024**	**0.002**	**0.007**	**< 0.001**	**0.002**	0.163	**< 0.001**	**< 0.001**
	T x D	**0.007**	**0.002**	0.475	0.884	0.995	0.403	0.512	**0.005**	0.056	0.137	0.970	**0.022**
Chlorophyll index	T	0.158	0.085	0.156	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.002**	0.112
	D	0.815	**< 0.001**	**0.020**	**0.007**	**0.010**	**< 0.001**	0.102	**< 0.001**	**< 0.001**	**< 0.001**	**0.010**	0.067
	T x D	0.711	0.586	0.874	0.283	**0.032**	**0.009**	0.573	0.118	0.062	0.617	0.937	0.875
A_{max}	T	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.019**	**< 0.001**	**< 0.001**	**0.004**	**< 0.001**	**< 0.001**	**< 0.001**
	D	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.143	**< 0.001**	**< 0.001**	**< 0.001**
	T x D	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.005**	**0.048**	0.181	**< 0.001**	0.439	**< 0.001**	**0.001**	**0.033**
G_{Smax}	T	**< 0.001**	**< 0.001**	**< 0.001**	0.077	**< 0.001**	0.292	**< 0.001**	**< 0.001**	**< 0.001**	**0.015**	**0.002**	**< 0.001**
	D	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
	T x D	0.110	0.667	**< 0.001**	**0.001**	0.501	0.388	0.062	**< 0.001**	0.329	0.053	0.581	0.164

response particularly to drought stress with the length of treatment (Fig. 5). Such a shift means that a significant effect of drought was manifested under longer treatments at lower temperatures. The results also revealed that the Tobak variety had less sensitivity to drought and high temperatures, particularly at DC 61. The differences in sensitivity between genotypes were more evident from the 3-day exposure to the temperature and drought stress treatment. The effect of growth stage was clearly evident from the interaction between temperature and drought, especially after 7 days of treatment. While at earlier growth stages, the steep decline of A_{max} with temperature under drought stress was observed, at DC 75, there was a strong reduction of A_{max}, which was also evident at lower temperatures. The effect of temperature alone (in well-watered control) was relatively low and, in most cases, statistically insignificant. While in the Bohemia variety, there was a significant drop of A_{max} with high temperature (38 °C), which was observed only at the late growth stage (DC 75); in Tobak, this result was observed only at DC 31. G_{Smax} exhibited a similar pattern of response to high temperatures but a much stronger response to drought stress (Fig. 6). A higher sensitivity of G_{Smax} to drought was evident from higher differences between the control and drought treatments in the 3-day treatment and from higher effects of drought at lower temperatures. Such differences resulted in generally less evident and mostly insignificant interactions between drought and temperature effects. Significant interactions were found only in the Bohemia variety at DC 61 and the Tobak variety at DC 31 (7 days). A negative effect of temperature on stomatal conductance under the control (well-watered) treatment was found only in the Tobak variety at DC 31.

3.5. Relationships to heat degree-days

In order to integrate the effect of length of heat stress from 3 and 7-day temperature treatment, we analyzed the relationships of heat degree-days (HDD) to A_{max} and HDD to GW (Figs. 7 and 8). Such relationships show trends in the effect of temperature on main physiological and production parameters better then relationships using simply length of the treatment. These results demonstrate that under combined drought and high temperature stress is A_{max} reduced already in lower HDD in variety Bohemia as compared to variety Tobak. There was negligible decrease of A_{max} until HDD around 5 in variety Tobak, while in variety Bohemia was decline of A_{max} observable from HDD of approximately 1. These relationships also showed that growth stage modulates response of Tobak plants to drought. While drought effect at stage DC 61 was found only in HDD above 5, at growth stages DC 31 and DC 75 the drought effect was evident already without additional heat stress (HDD = 0). However, the further decline of A_{max} with increasing HDD was similarly at all growth stages demonstrated for HDD values above 5. For the well-watered control the effect of HDD on A_{max} was very small in both varieties. While a slight A_{max} decline was evident only at DC 75 in variety Bohemia, it was also found at DC 31 in variety Tobak. On the contrary to A_{max} the relationships between HDD and GW didn't show interactions between heat stress (HDD) and drought. In both varieties and all growth stages the pattern of the relationship was very similar for well-watered control and drought-stressed plants; however, GW values were slightly reduced under drought stress. The effect of heat stress on GW was relatively low and in most cases observable for HDD values up to 5. Only in variety Tobak and growth stage DC 61 the heat stress effect was found for higher HDD values. In variety Bohemia at DC 31 an increase of GW was found in the range of HDD between 0 and 5.

4. Discussion

Drought and high temperature often occur simultaneously, but their effects on crop growth, physiology and productivity are usually investigated individually, and the understanding of mutual interactions is still poor (Prasad et al., 2008). Simultaneous occurrence of heat and drought stress increases the deleterious effect, such that the effect considerably exceeds the simple additive effect of individual factors (synergistic interaction; Pradhan et al., 2012; Shah and Paulsen, 2003). Plants under combined heat and drought stress show reduced growth, accelerated senescence and earlier death as compared to plants exposed to single stress (Machado and Paulsen, 2001). Thus, the duration of combined drought and heat stress is likely crucial for resulting interactive effect (Meehl and Tebaldi, 2004), as serious detrimental effects associated with irreversible changes occur beyond the certain tipping point. This non-linearity of response based on stress duration makes the predictions of impacts of combined stress even more difficult. On the other hand, under certain circumstances such as short duration of

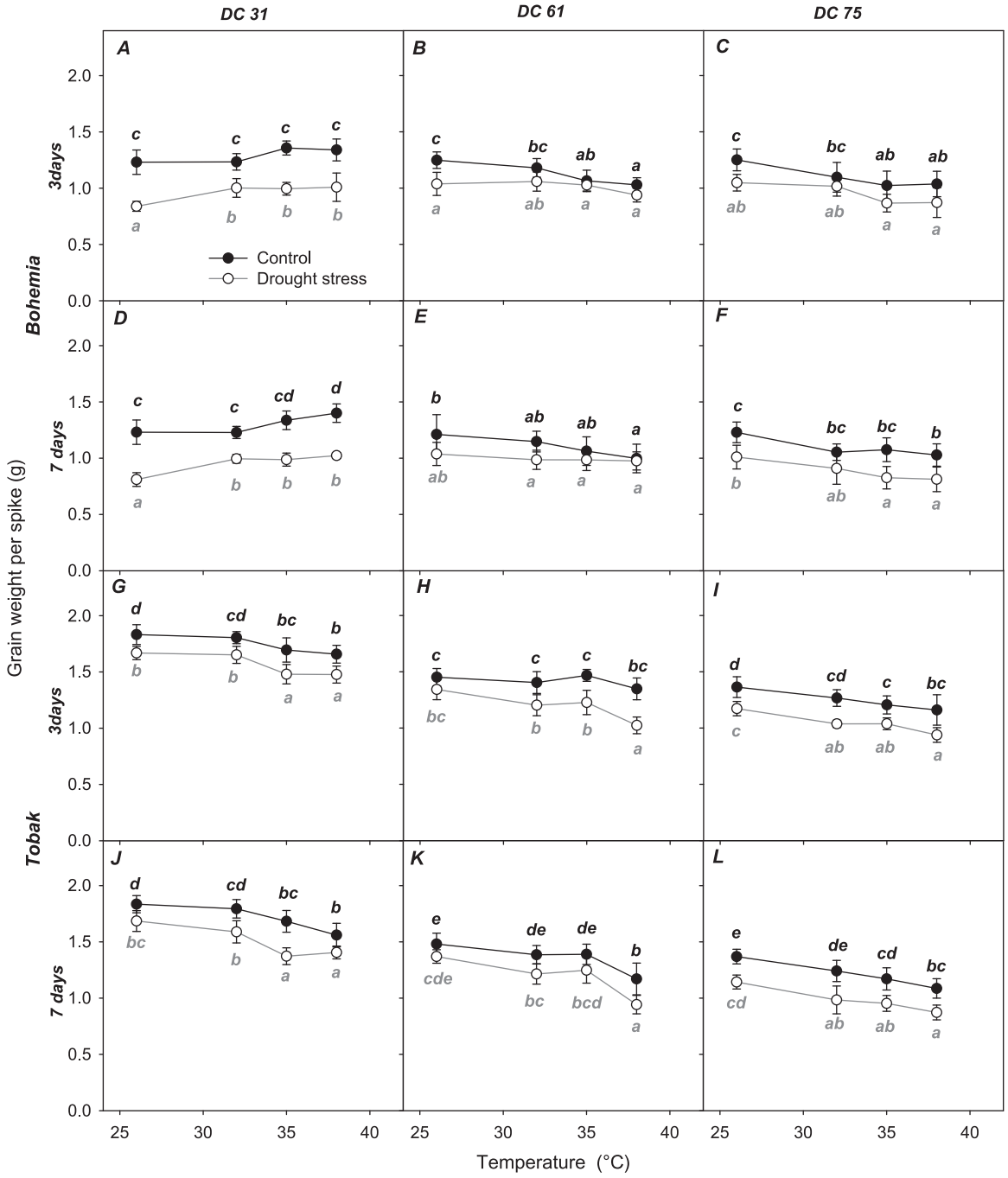

Fig. 2. Grain weight (g) per main spike in winter wheat varieties Bohemia (A–F) and Tobak (G–L) under exposure to temperature and drought stress treatments for 3 (A–C, G–I) and 7 (D–F, J–L) days at growth stages DC 31–beginning of stem elongation (A, D, G, J), DC 61–beginning of anthesis (B, E, H, K) and DC 75–medium milk ripe (C, F, I, L). Means (points) and standard deviations (error bars) are presented (n = 7). Different letters denote statistically significant differences among means tested by ANOVA with Tukey's post hoc test (P = 0.05) within each variety, growth stage and length of treatment separately.

combined stress or temperature rise bellow chronic stress limit, one environmental factor may alleviate the effect of second stress factor through several mechanisms, for example through drought escape (Wardlaw, 2002) or increased water use efficiency (Urban et al. this issue). As the most studies focused on combined drought and heat stress lasted more than two weeks (e.g. Shah and Paulsen, 2003; Nicolas et al., 1984), in our work we aimed to evaluate the impacts of short-term drought and heat stress (3 and 7 days) on wheat physiology and yield formation. We assumed that length of combined drought and heat stress around 7 days represent a tipping point for a detrimental effect on plant photosynthesis and in certain growth stages also on yield formation. While drought stress tends to occur for relatively longer periods compared to temperature the heat stress episodes under present climate rarely exceed several days within the growing season of wheat (e.g. Trnka et al., 2014).

There is a number of processes that can be affected by interactions between drought stress and high temperatures such as leaf development, stomatal conductance, photosynthetic processes, abortion of kernels, synthesis of starch, acceleration of senescence, chlorophyll degradation, etc. (reviewed by Lipiec et al., 2013). So far, there is still a lack of knowledge not only of the response of individual processes but also of the effects on production parameters that are crucial for reliable predictions of climate change impacts. One of the most frequently reported wheat responses to heat stress, particularly at the anthesis, is the

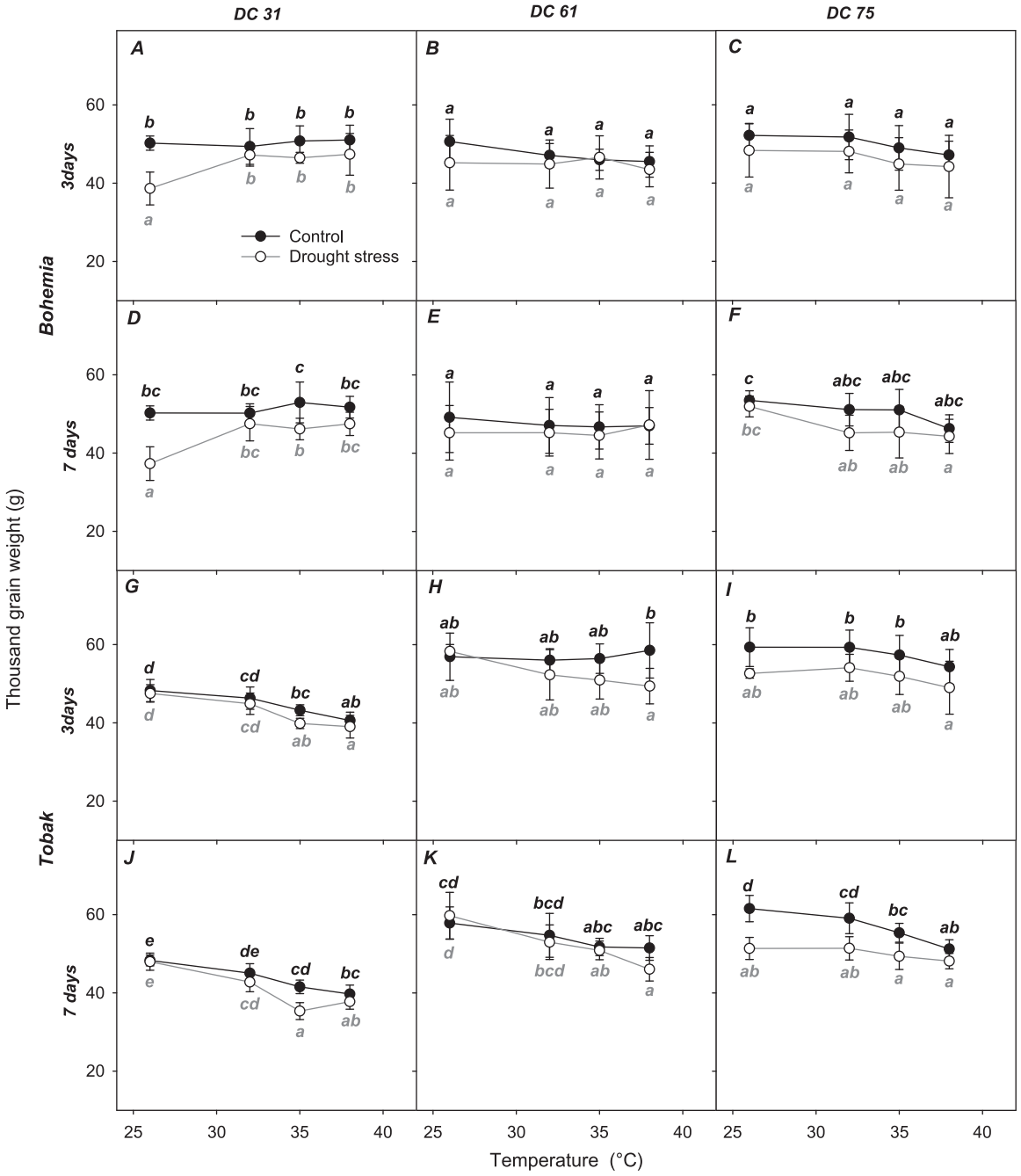

Fig. 3. Thousand grain weight in winter wheat varieties Bohemia (A–F) and Tobak (G–L) under exposure to temperature and drought stress treatments for 3 (A–C, G–I) and 7 (D–F, J–L) days at growth stages DC 31–beginning of stem elongation (A, D, G, J), DC 61–beginning of anthesis (B, E, H, K) and DC 75–medium milk ripe (C, F, I, L). Means (points) and standard deviations (error bars) are presented (n = 7). Different letters denote statistically significant differences among means tested by ANOVA with Tukey's post hoc test (P = 0.05) within each variety, growth stage and length of treatment separately.

reduction of GN in spikes. As a result of heat stress, parthenocarpy, abortion and shrinking of kernels occurs shortly after anthesis (Tashiro and Wardlaw, 1990). Most results, however, are based on longer exposures to higher temperatures (reviewed by Farooq et al., 2011). In this particular study, the duration of combined heat and drought stress reflected more likely conditions in Central Europe in this decade up to approximately 2040. Despite ongoing climate change, temperatures over 35 °C, especially at the DC 31 and DC 61 growth stages, are and will be rare also due to the expected phenological shift (e.g. Trnka et al., 2015). In our study, the short-term effects of high temperatures negatively affected GN only at growth stage DC 61 and at the highest temperature (38 °C). This effect was also more pronounced in the Tobak variety originating from Germany, while the Czech variety Bohemia showed less sensitivity in this parameter. In general, however, the number of grains was affected very slightly by short-term exposure to both drought and high temperature stress. As reported by Farooq et al. (2011) the optimum and maximum temperatures increase with crop ontogeny. In our study, the negative effect at the early growth stage (DC 31) was a response to drought stress rather than higher temperatures, even though the plants should have a lower temperature optimum. Our results even indicated that there was a small positive effect of higher temperatures on GN and on GW at the early growth stage (DC 31). As the ability of compensation between yield components is at early growth stages more important, understanding of mechanisms behind this positive effect on GW is difficult. One of possible mechanisms can be effect of higher temperatures on accelerated reduction of non-

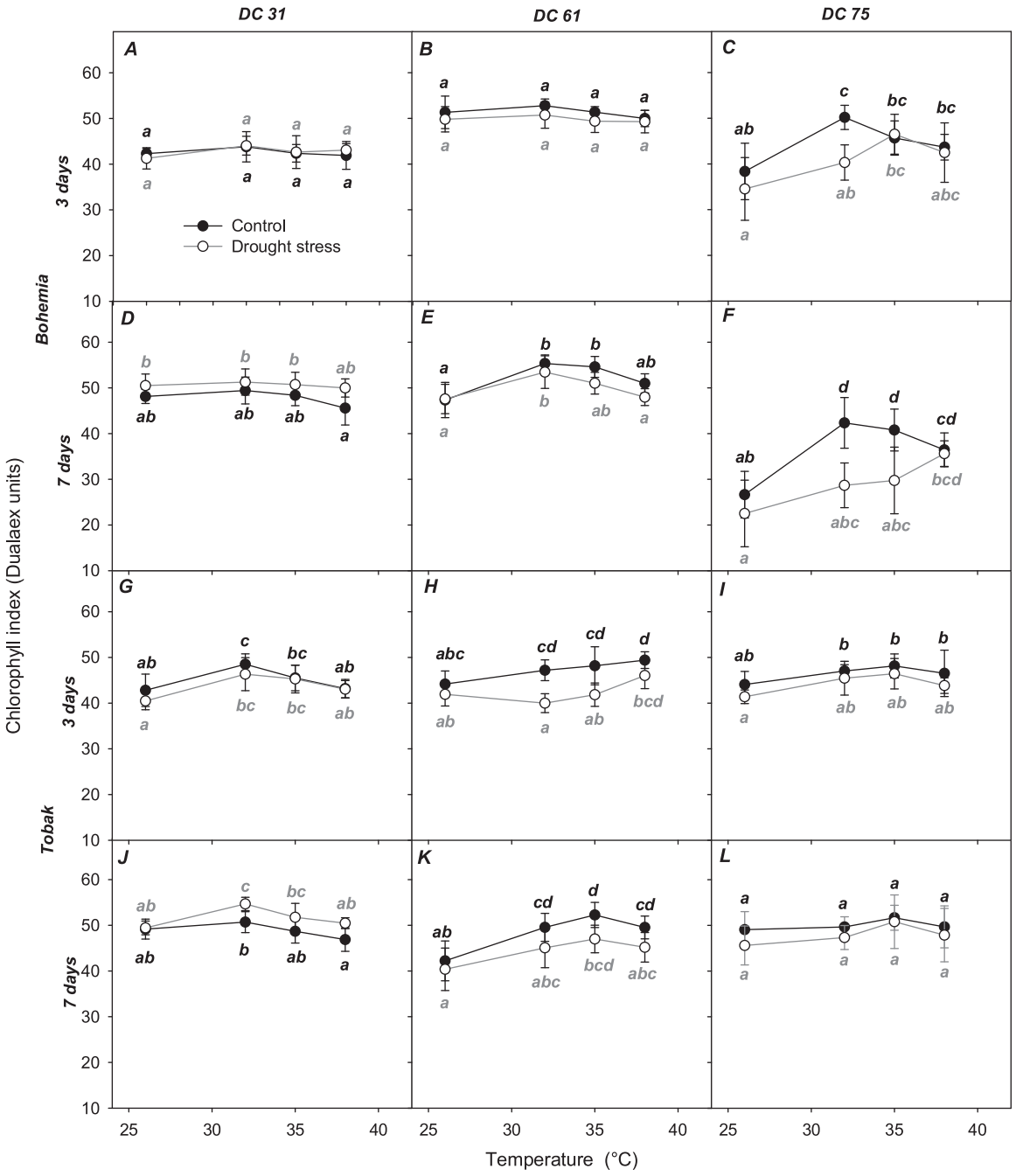

Fig. 4. Chlorophyll index (measured *in vivo* using the instrument Dualex 4 Flav) in winter wheat varieties Bohemia (A–F) and Tobak (G–L) under exposure to temperature and drought stress treatments for 3 (A–C, G–I) and 7 (D–F, J–L) days at growth stages DC 31–beginning of stem elongation (A, D, G, J), DC 61–beginning of anthesis (B, E, H, K) and DC 75–medium milk ripe (C, F, I, L). Means (points) and standard deviations (error bars) are presented (n = 7). Different letters denote statistically significant differences among means tested by ANOVA with Tukey's post hoc test (P = 0.05) within each variety, growth stage and length of treatment separately.

productive tillers, resulting in less competition for resources and compensatory effects on GN (Valério et al., 2013). Although temperature optima during stem elongation are lower than optima at later growth stages, the temperature maxima may be even higher due to high compensation ability (Porter and Gawith, 1999). However, the most commonly reported effect of heat stress during stem elongation is negative, due to shortening of flowering initiation and resulting lower number of spikelets per spike (reviewed by Barnabas et al., 2008). On the contrary, negative effect of heat stress on GN was most obvious at DC 61, although these changes were also relatively small. Unlike our results, Ugarte et al. (2007) reported the highest effect of elevated temperatures on wheat yield parameters during early growth stages (stem elongation), whereas at anthesis, the effect was the lowest. These opposite results may be explained by the duration of elevated temperature exposure. It can be assumed that in the period of intense growth (stem elongation), the duration of heat stress is critical, whereas at anthesis, which is relatively short, the temperature itself is crucial. Heat stress after anthesis can affect the number of grains in a spike only to a limited extent and reduces the grain filling in particular, resulting in lower GW (Dias and Lidon, 2009). However, the length of exposure to high temperatures may also be critical for grain filling. The effect of high temperatures during grain filling is associated with higher respiration (Spiertz, 1974) and induced senescence (Hays et al., 2007). For the effect of increased respiration to be reflected in GW, the duration of heat stress has to be longer, whereas the induction of senescence is more related to temperature and genotype (Hays et al., 2007). In order

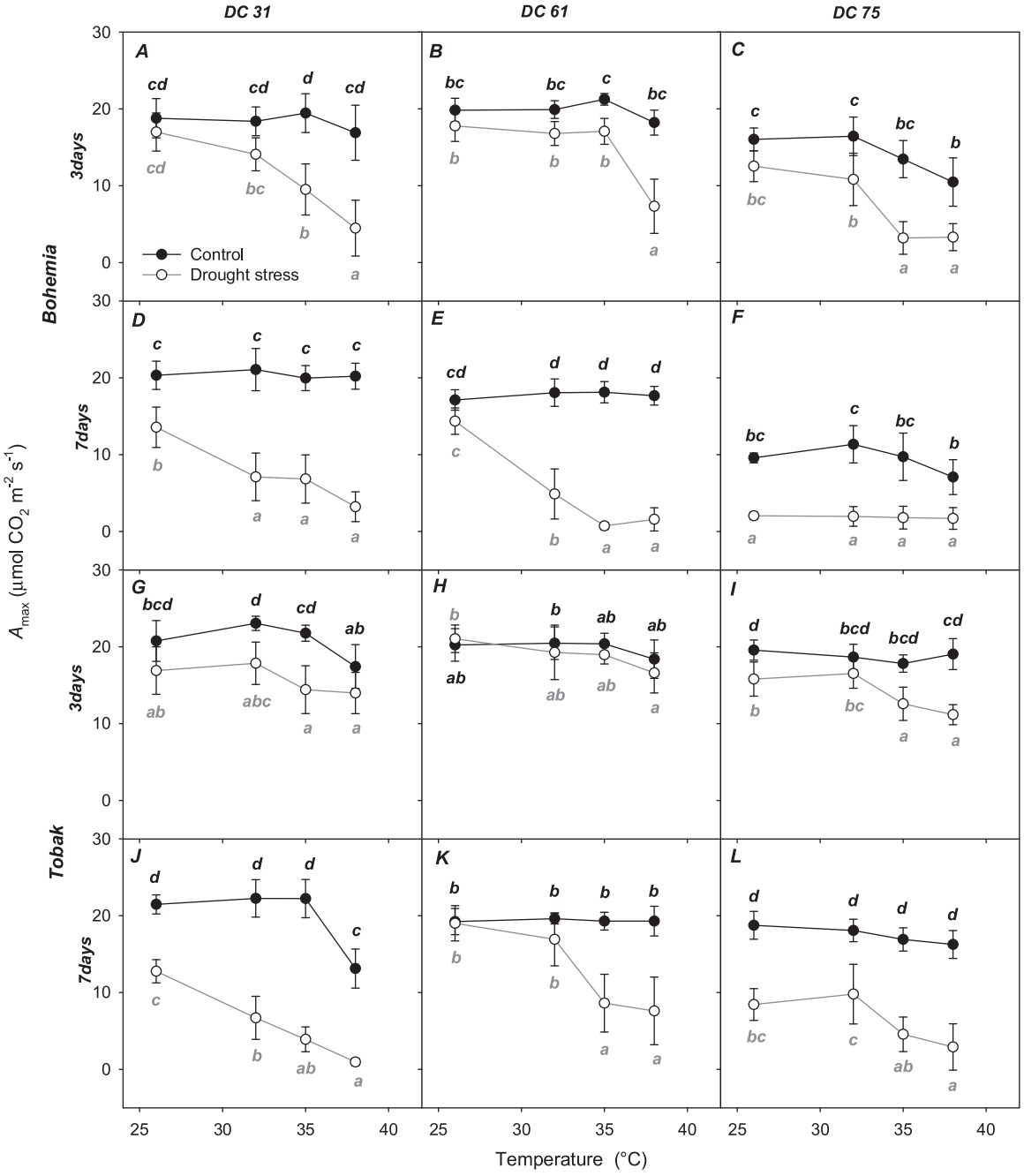

Fig. 5. Light saturated CO_2 assimilation rate (A_{max}) in winter wheat varieties Bohemia (A–F) and Tobak (G–L) under exposure to temperature and drought stress treatments for 3 (A–C, G–I) and 7 (D–F, J–L) days at growth stages DC 31–beginning of stem elongation (A, D, G, J), DC 61–beginning of anthesis (B, E, H, K) and DC 75–medium milk ripe (C, F, I, L). Means (points) and standard deviations (error bars) are presented (n = 7). Different letters denote statistically significant differences among means tested by ANOVA with Tukey's post hoc test (P = 0.05) within each variety, growth stage and length of treatment separately.

to compare heat periods with different lengths and intensities, so called thermal indices were introduced such as heat degree-days (HDD; Liu et al., 2014) or high temperature index (HTI; Rane and Nagarajan, 2004). Due to best description of spatial variability and higher correlation to the heat stress yield response, Liu et al. (2014) suggested HDD as the most suitable heat stress index for wheat. Thus, we used HDD to integrate length and intensity of heat stress and to analyze relationships to main physiological and production parameters A_{max} and GW, respectively. As the relationships between HDD and A_{max} or GW are represented by more points in comparison to simple maximum temperature used, these describe better the trends of response to heat stress, although non-linear response to HDD was found for both A_{max} and GW. The problem of using HDD may be a comparison of heat effect at different growth stages. We used HDD for temperature maximum of 30 °C reported for anthesis (Porter and Gawith, 1999). However, the temperature maxima for grain filling lies between 30 and 40 °C and for spikelet initiation, which is almost identical with growth stage DC 31, between 25 and 30 °C (reviewed by Porter and Gawith, 1999). On the other side, these authors also reported temperature maxima for stem elongation > 40 °C. Such contradictory results imply that temperature thresholds for beginning of stem elongation and grain filling are ambiguous. Therefore, we used the same threshold of 30 °C to calculate HDD for all growth stages and based on the fact that no shift of relationships was found between individual growth stages we can infer that HDD based on 30 °C threshold can be used to evaluate heat stress at all three growth stages studied. Our results, showing HDD increase by

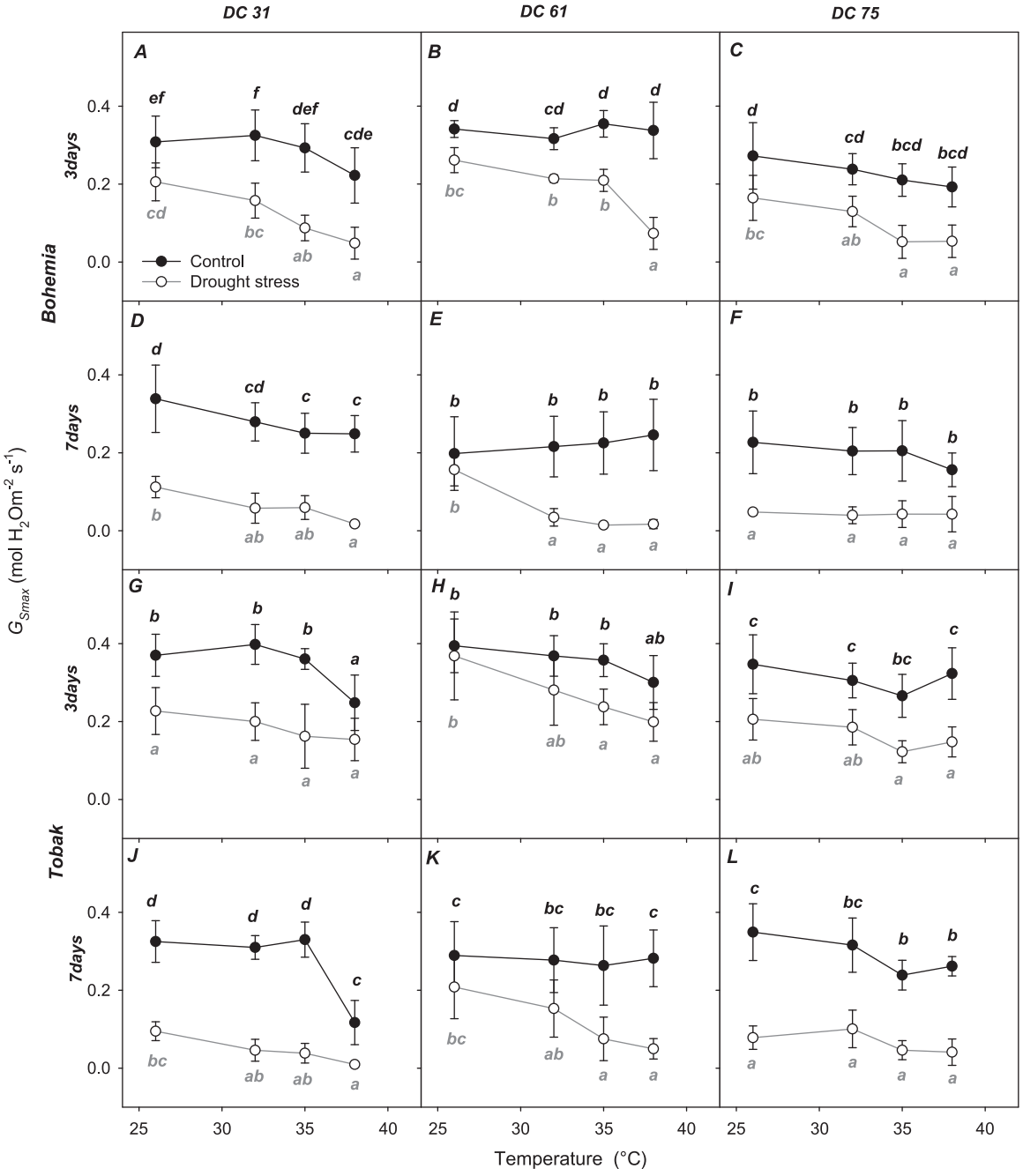

Fig. 6. Light saturated stomatal conductance (G_{Smax}) in winter wheat varieties Bohemia (A–F) and Tobak (G–L) under exposure to temperature and drought stress treatments for 3 (A–C, G–I) and 7 (D–F, J–L) days at growth stages DC 31–beginning of stem elongation (A, D, G, J), DC 61–beginning of anthesis (B, E, H, K) and DC 75–medium milk ripe (C, F, I, L). Means (points) and standard deviations (error bars) are presented (n = 7). Different letters denote statistically significant differences among means tested by ANOVA with Tukey's post hoc test (P = 0.05) within each variety, growth stage and length of treatment separately.

11 and GW reduction by 10–15%, corresponds to results of Liu et al. (2014) who reported drop of grain yield by 1 t ha^{-1} for HDD increase by 40.

Chlorophyll content is often used as an indicator of drought stress response or tolerance (e.g., Li et al., 2006; Arjenaki et al., 2012; Mohammadi et al., 2009). Some of studies also show similar effects of heat stress (Ristic et al., 2007) or combined heat and temperature stress (Mohammadi et al., 2009). Loss of chlorophyll under heat stress is strongly correlated with heat-induced damage of thylakoid membranes. Reduced water availability can induce a decline in membrane stability as well (Tuba et al., 1996), and the simultaneous action of both factors may have an additive effect resulting in the decline of chlorophyll content. On the other hand, short-term or mild drought stress can induce morphological changes, which leads to increased leaf thickness (Poorter et al., 2009; Lamont et al., 2002). These changes may result in the temporary increase of chlorophyll content per area unit, which we also observed in our experiment particularly for the early growth stage (DC 31). In later growth stages, the drought and heat stress can induce accelerated senescence as a mechanism for plant survival (Munné-Bosch and Alegre, 2004). Our results showed considerable effects of variety on the drought-/heat-induced decline of chlorophyll content at growth stage DC 75. Senescence is more strongly induced by drought in the variety Bohemia. Many studies indicated that genotypes with delayed senescence (stay green) have improved yield and transpiration efficiency under water-limited conditions (e.g., Verma et al., 2004; Haussmann et al., 2002). The ability to maintain higher chlorophyll

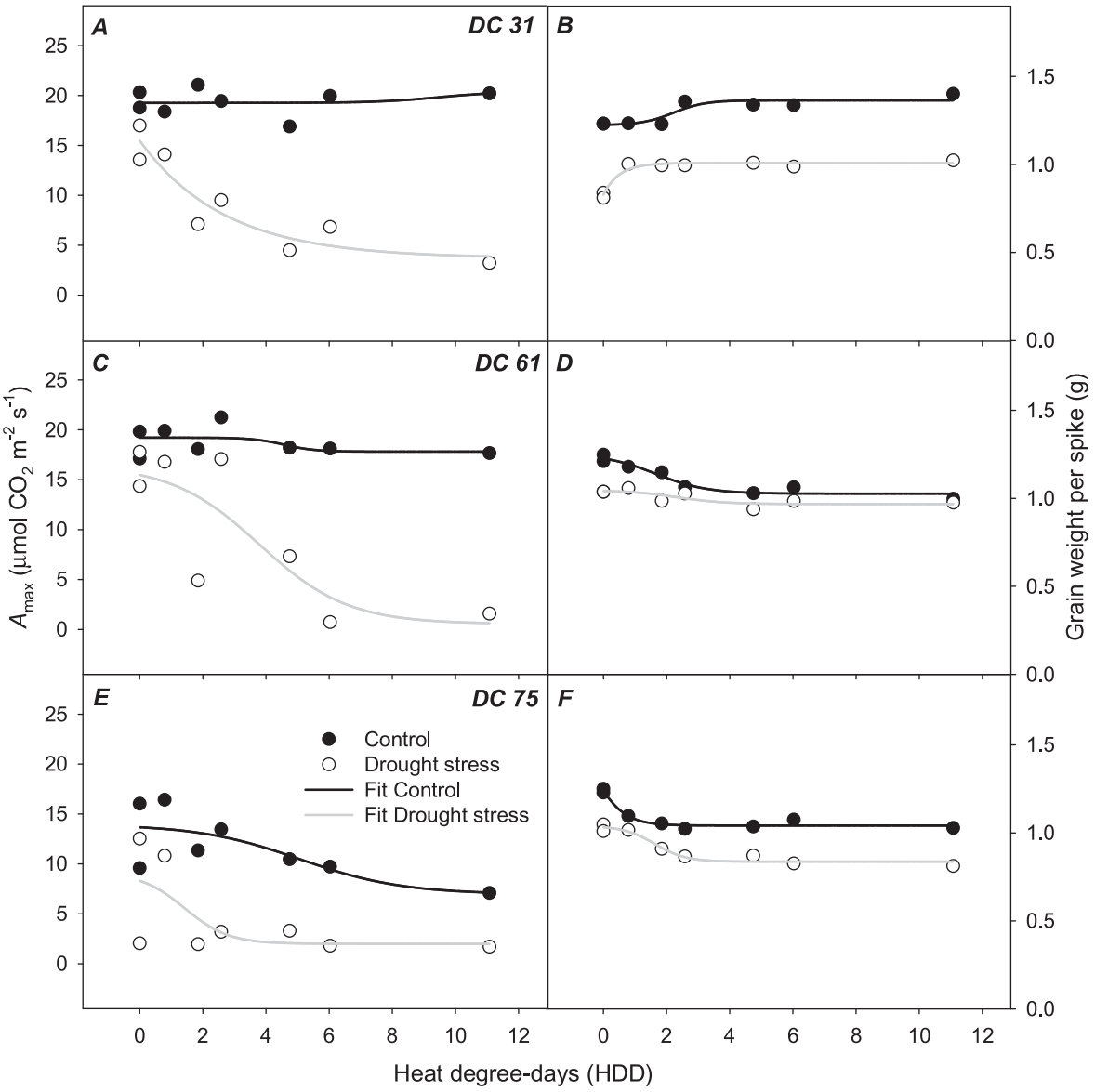

Fig. 7. Relationships between additional heat stress during experimental treatment expressed in heat degree-days (HDD) and light saturated CO_2 assimilation rate (A_{max}; A, C, E) or grain weight per spike (B, D, F) at growth stages DC 31–beginning of stem elongation (A, B), DC 61–beginning of anthesis (C, D) and DC 75–medium milk ripe (E, F) in variety Bohemia. Means (points) from each treatment for both treatment lengths (3 and 7 days) are presented. The sigmoid function was fitted to the data of control and drought stress treatment separately.

content under drought stress can thus be utilized as a reliable indicator of drought tolerance in different genotypes (Li et al., 2006).

Our results revealed early (3 days of exposure) responses of photosynthetic parameters to high temperature and drought stress and clear interactions between these two factors, which resulted in multiplicative effects of high temperature on drought responses. Although this response was only slightly reflected in final yield parameters, probably due to fast photosynthetic recovery and compensatory mechanisms in wheat, A_{max} and G_{Smax} can serve as very useful indicators of the dynamic response to drought and high temperature stress.

The differences in A_{max} and G_{Smax} responses can also indicate the stomatal and biochemical limitations of photosynthesis, which also help to separate the effect of drought and temperature (Flexas et al., 2004). Moreover, the stomatal response can be sensed remotely using infrared thermal imaging as an indicator of drought tolerance (Klem et al., 2017). The stomatal response to drought and to combined heat and drought stress is very fast and under short-term exposure, rapidly returns to its original state (Miyashita et al., 2005). On the other hand, biochemical limitations of photosynthesis may be a result of deeper damage, and the recovery back to the original state is thus usually slower or even impossible (Chaves et al., 2009). Based on the relationship between HDD and A_{max} it can be also assumed that HDD with temperature threshold 30 °C is a suitable heat stress indicator of photosynthetic response. However, high variability of A_{max} under drought stress shows an apparent effect of drought stress length which is not included in HDD. Almost no shift of the relationships between HDD and A_{max} with growth stages was observed, indicating similar temperature threshold for all stages. Our results show a significant interaction between temperature and drought stress, particularly in the A_{max} response. Although in our short-term experiment, this interaction was not manifested significantly in yield formation parameters, under longer exposure, a similar response pattern can be expected in yield parameters and hence the additive or multiplicative effect of temperature on the effects of drought stress and vice versa (Jiang and Huang, 2000) as confirmed by Urban et al. (this issue). As Rötter et al. (this issue) noted, studies considering both temperature and drought are still rare and are essential for the improvement of the crop models. The tools used in the present study are the best currently available tools enabling a

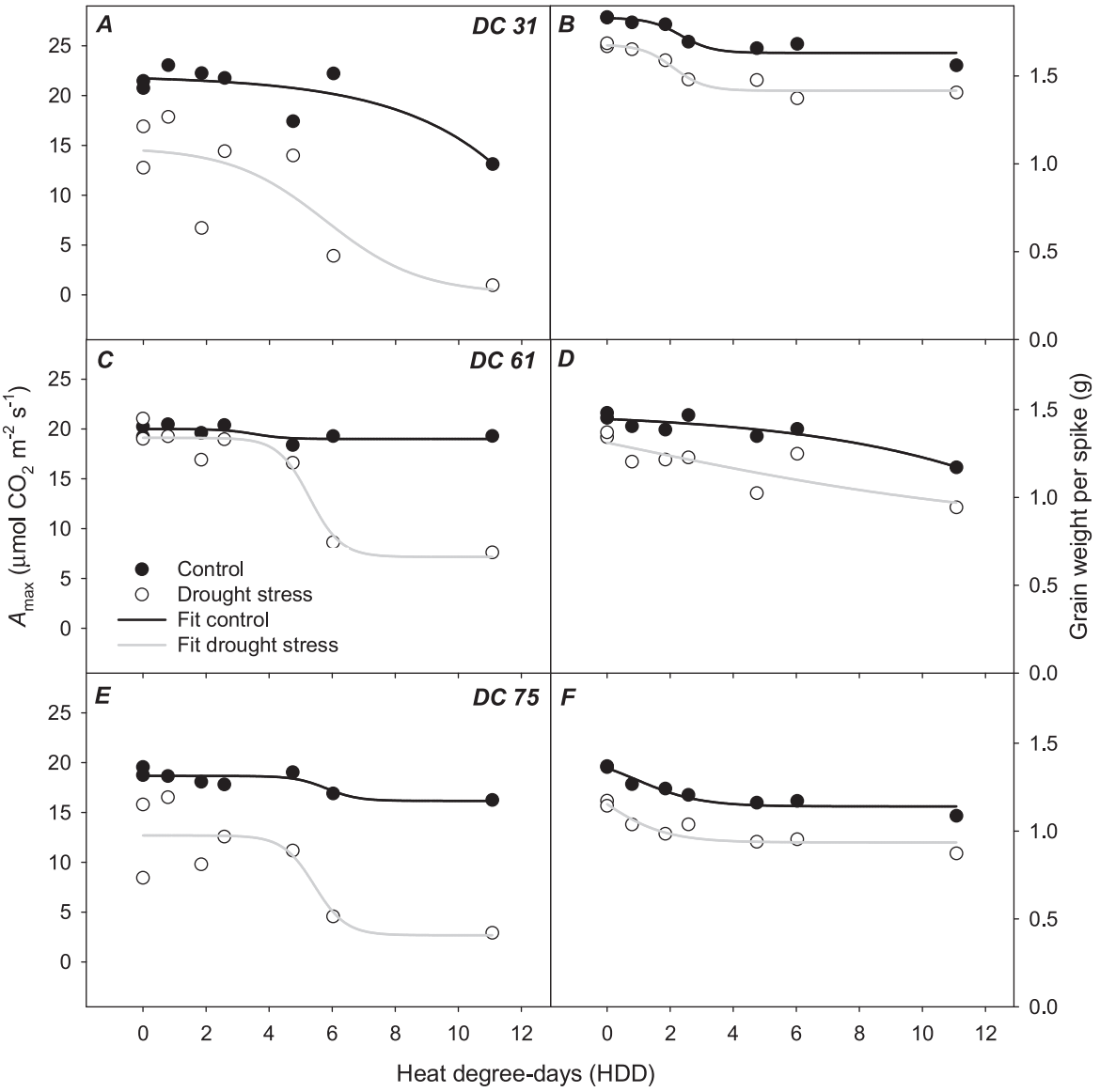

Fig. 8. Relationships between additional heat stress during experimental treatment expressed in heat degree-days (HDD) and light saturated CO_2 assimilation rate (A_{max}; A, C, E) or grain weight per spike (B, D, F) at growth stages DC 31–beginning of stem elongation (A, B), DC 61–beginning of anthesis (C, D) and DC 75–medium milk ripe (E, F) in variety Tobak. Means (points) from each treatment for both treatment lengths (3 and 7 days) are presented. The sigmoid function was fitted to the data of control and drought stress treatment separately.

generalized estimate of the impact of various factors, e.g., increased heat stress (Asseng et al., 2015), on crops at the national or even global scale, where considerable uncertainties remain (e.g., Asseng et al., 2013), and the topic is under close scrutiny (e.g., White et al., 2017). Data on the drought and heat stress interaction are essential for further model development, and the critical importance of new experimental data can be documented by the impact that experimental datasets (Asseng et al., 2015; Martre et al., 2017) are having on the modeling community.

5. Conclusions

The results of this study showed that short-term heat and drought stress, and particularly their combination, are manifested in additive or multiplicative interactive effects on photosynthetic parameters (namely, the rate of CO_2 assimilation rate), but the manifestation of this response in yield parameters is relatively small. This means that the short-term (up to 7 days) effects of these stress factors are eliminated by the recovery of photosynthetic processes and likely also by compensation of yield parameters. However, despite this lower effect on yield, certain trends in the response to heat and drought stress can be derived. (1) The negative effect of drought on GW was highest in variety Bohemia at the early growth stage (DC 31) and decreased at later stages, while in variety Tobak the drought effect was smaller and remained similar in all growth stages. (2) More specifically, drought affected GN particularly in variety Bohemia at DC 31 and TGW in variety Tobak at DC 75. (3) The effects of high temperature on yield parameters were more pronounced at later stages and particularly at DC 61. (4) The effect of drought stress on chlorophyll content was manifested in variety Bohemia at the growth stage DC 75 and in variety Tobak at DC 61. (5) The response of photosynthetic parameters to both drought and heat stress was more pronounced as compared to yield parameters and showed also significant change with length of stress. (6) We have demonstrated the potential of HDD to integrate length and intensity of heat stress at different growth stages, particularly for an estimation of effects on yield parameters.

Acknowledgements

The research was financially supported by the National

Sustainability Program I (NPU I), grant number LO1415 with the support of the Specific Research Grants, provided by the Ministry of Education, Youth and Sports of the Czech Republic. The infrastructure used within this project was supported by the projects CzeCOS (LM2015061) and CzeCOS Proces (CZ.02.1.01/0.0/0.0/16_013/0001609).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.fcr.2018.02.022.

References

Al-Khatib, K., Paulsen, G.M., 1984. Mode of high temperature injury to wheat during grain development. Physiol. Plant. 61, 363–368.

Arjenaki, F.G., Jabbari, R., Morshedi, A., 2012. Evaluation of drought stress on relative water content: chlorophyll content and mineral elements of wheat (*Triticum aestivum* L.) varieties. Int. J. Agric. Crop Sci. 4, 726–729.

Asseng, S., Foster, I.A.N., Turner, N.C., 2011. The impact of temperature variability on wheat yields. Glob. Change Biol. 17, 997–1012.

Asseng, S., Ewert, F., Rosenzweig, C., Jones, J.W., Hatfield, J.L., Ruane, A.C., Boote, K.J., Thorburn, P.J., Rötter, R.P., Cammarano, D., Brisson, N., Basso, B., Martre, P., Aggarwal, P.K., Angulo, C., Bertuzzi, P., Biernath, C., Challinor, A.J., Doltra, J., Gayler, S., Goldberg, R., Grant, R., Heng, L., Hooker, J., Hunt, L.A., Ingwersen, J., Izaurralde, R.C., Kersebaum, K.C., Müller, C., Naresh Kumar, S., Nendel, C., O'Leary, G., Olesen, J.E., Osborne, T.M., Palosuo, T., Priesack, E., Ripoche, D., Semenov, M.A., Shcherbak, I., Steduto, P., Stöckle, C., Stratonovitch, P., Streck, T., Supit, I., Tao, F., Travasso, M., Waha, K., Wallach, D., White, J.W., Williams, J.R., Wolf, J., 2013. Uncertainty in simulating wheat yields under climate change. Nat. Clim. Change 3 (9), 827–832.

Asseng, S., Ewert, F., Martre, P., Rötter, R.P., Lobell, D.B., Cammarano, D., Kimball, B.A., Ottman, M.J., Wall, G.W., White, J.W., Reynolds, M.P., Alderman, P.D., Prasad, P.V.V., Aggarwal, P.K., Anothai, J., Basso, B., Biernath, C., Challinor, A.J., De Sanctis, G., Doltra, J., Fereres, E., Garcia-Vila, M., Gayler, S., Hoogenboom, G., Hunt, L.A., Izaurralde, R.C., Jabloun, M., Jones, C.D., Kersebaum, K.C., Koehler, A.-K., Müller, C., Naresh Kumar, S., Nendel, C., O'Leary, G., Olesen, J.E., Palosuo, T., Priesack, E., Eyshi Rezaei, E., Ruane, A.C., Semenov, M.A., Shcherbak, I., Stöckle, C., Stratonovitch, P., Streck, T., Supit, I., Tao, F., Thorburn, P.J., Waha, K., Wang, E., Wallach, D., Wolf, J., Zhao, Z., Zhu, Y., 2015. Rising temperatures reduce global wheat production. Nat. Clim. Change 5 (2), 143–147.

Barnabas, B., Jager, K., Feher, A., 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 31, 11–38.

Bilal, M., Rashid, R.M., Rehman, S.U., Iqbal, F., Ahmed, J., Abid, M.A., Ahmed, Z., Hayat, A., 2015. Evaluation of wheat genotypes for drought tolerance. J. Green Physiol. Genet. Genom. 1, 11–21.

Bita, C.E., Gerats, T., 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front. Plant Sci. 4, 273.

Bolhar-Nordenkampf, H.R., Hofer, M., Lechner, E.G., 1991. Analysis of light induced reduction of the photochemical capacity in field-grown plants: evidence for photoinhibition. Photosynth. Res. 27, 31–39.

Calderini, D.F., Abeledo, L.G., Savin, R., Slafer, G.A., 1999. Effect of temperature and carpel size during pre-ATS on potential grain weight in wheat. J. Agric. Sci. (Cambridge) 132, 453–460.

Camejo, D., Rodríguez, P., Morales, M.A., Del'Amico, J.M., Torrecillas, A., Alarcón, J.J., 2005. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J. Plant Physiol. 162 (3), 281–289.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103 (4), 551–560.

Demirevska, K., Simova-Stoilova, L., Vassileva, V., Feller, U., 2008. Rubisco and some chaperone protein responses to water stress and rewatering at early seedling growth of drought sensitive and tolerant wheat varieties. Plant Growth Regul. 56, 97–106.

Dias, A.S., Lidon, F.C., 2009. Evaluation of grain filling rate and duration in bread and durum wheat, under heat stress after anthesis. J. Agron. Crop Sci. 195, 137–147.

Farooq, M., Bramley, H., Palta, J.A., Siddique, K.H., 2011. Heat stress in wheat during reproductive and grain-filling phases. Crit. Rev. Plant Sci. 30 (6), 491–507.

Ferris, R., Ellis, R.H., Wheeler, T.R., Hadley, P., 1998. Effect of high temperature stress at ATS on grain yield and biomass of field-grown crops of wheat. Ann. Bot. 82, 631–639.

Flexas, J., Bota, J., Cifre, J., Mariano Escalona, J., Galmés, J., Gulías, J., Lefi, El-Kadri, Martínez-Cañellas, S.F., Moreno, M.T., Ribas-Carbó, M., Riera, D., Sampol, B., Medrano, H., 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. Ann. Appl. Biol. 144 (3), 273–283.

Flexas, J., Diaz-Espejo, A., Berry, J.A., Cifre, J., Galmés, J., Kaldenhoff, R., Medrano, H., Ribas-Carbó, M., 2007. Analysis of leakage in IRGA's leaf chambers of open gas exchange systems: quantification and its effects in photosynthesis parameterization. J. Exp. Bot. 58 (6), 1533–1543.

Gibson, L.R., Paulsen, G.M., 1999. Yield components of wheat grown under high temperature stress during reproductive growth. Crop Sci. 39, 1841–1846.

Hasanuzzaman, M., Hossain, M.A., Teixeira da Silva, J.A., Fujita, M., 2012. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Bandi, V., Shanker, A.K., Shanker, C., Mandapaka, M. (Eds.), Crop Stress and Its Management: Perspectives and Strategies. Springer, The Netherlands, pp. 261–315.

Haussmann, B., Mahalakshmi, V., Reddy, B., Seetharama, N., Hash, C., Geiger, H., 2002. QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor. Appl. Genet. 106, 133–142.

Hays, D.B., Do, J.H., Mason, R.E., Morgan, G., Finlayson, S.A., 2007. Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar. Plant Sci. 172, 1113–1123.

Hirschi, M., Seneviratne, S.I., Alexandrov, V., Boberg, F., Boroneant, C., Christensen, O.B., Formayer, H., Orlowsky, B., Stepanek, P., 2011. Observational evidence for soil-moisture impact on hot extremes in southeastern Europe. Nat. Geosci. 4 (1), 17–21.

Hossain, A., Lozovskaya, M.V., Zvolinsky, V.P., Tutuma, N.V., 2012. Effect of soil resources and climatic factors (temperature) on spring wheat and barley in the northern Bangladesh and southern Russia. In: Paper Presented in "International Scientific and Practical Conference on Problems of Environmental Management and Conservation of Ecological Balance in the Arid Zones". "Caspian Scientific Research Institute of Arid Agriculture", Salt Zaymische, Chorniarsky district, Astrakhan State, Russia, from 16 to 18 May, 2012.

Jiang, Y., Huang, B., 2000. Effects of drought or heat stress alone and in combination on Kentucky bluegrass. Crop Sci. 40 (5), 1358–1362.

Klem, K., Mishra, K., Novotná, K., Rapantová, B., Hodaňová, P., Mishra, A., Kováč, D., Urban, O., 2017. Distinct growth and physiological responses of *Arabidopsis thaliana* natural accessions to drought stress and their detection using spectral reflectance and thermal imaging. Funct. Plant Biol. 44 (3), 312–323.

Kumar, P.V., Rao, V.U.M., Bhavani, O., Dubey, A.P., Singh, C.B., 2016. Effect of temperature and photothermal quotient on the yield components of wheat (*Triticum aestivum* L.) in Indo-Gangetic Plains of India. Expl. Agric. 52 (1), 14–35.

Lamont, B.B., Groom, P.K., Cowling, R.M., 2002. High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. Funct. Ecol. 16 (3), 403–412.

Lawlor, D.W., Cornic, G., 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 25 (2), 275–294.

Li, R.H., Guo, P.G., Michael, B., Stefania, G., Salvatore, C., 2006. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. Agric. Sci. China 5 (10), 751–757.

Li, Y., Ye, W., Wang, M., Yan, X., 2009. Climate change and drought: a risk assessment of crop-yield impacts. Clim. Res. 39 (1), 31–46.

Lipiec, J., Doussan, C., Nosalewicz, A., Kondracka, K., 2013. Effect of drought and heat stresses on plant growth and yield: a review. Int. Agrophys. 27, 463–477.

Liu, B., Liu, L., Tian, L., Cao, W., Zhu, Y., Asseng, S., 2014. Post-heading heat stress and yield impact in winter wheat of China. Glob. Change Biol. 20, 372–381.

Liu, B., Asseng, S., Liu, L., Tang, L., Cao, W., Zhu, Y., 2016. Testing the responses of four wheat crop models to heat stress at anthesis and grain filling. Glob. Change Biol. 22, 1890–1903.

Lobell, D.B., Hammer, G.L., Chenu, K., Zheng, B., McLean, G., Chapman, S.C., 2015. The shifting influence of drought and heat stress for crops in northeast Australia. Glob. Change Biol. 21, 4115–4127.

Machado, S., Paulsen, G.M., 2001. Combined effects of drought and high temperature on water relations of wheat and sorghum. Plant Soil 233 (2), 179–187.

Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, Y., 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust. J. Crop Sci. 4, 580–585.

Martre, P., Reynolds, M.P., Asseng, S., Ewert, F., Alderman, P.D., Cammarano, D., Maiorano, A., Ruane, A.C., Aggarwal, P.K., Anothai, J., Basso, B., Biernath, C., Challinor, A.J., De Sanctis, G., Doltra, J., Dumont, B., Fereres, E., Garcia-Vila, M., Gayler, S., Hoogenboom, G., Hunt, L.A., Izaurralde, R.C., Jabloun, M., Jones, C.D., Kassie, B.T., Kersebaum, K.C., Koehler, A.K., Müller, C., Kumar, S.N., Liu, B., Lobell, D.B., Nendel, C., O'Leary, G., Olesen, J.E., Palosuo, T., Priesack, E., Eyshi Rezaei, E., Ripoche, D., Rötter, R.P., Semenov, M.A., Stöckle, C., Stratonovitch, P., Streck, T., Supit, I., Tao, F., Thorburn, P., Waha, K., Wang, E., White, J.W., Wolf, J., Zhao, Z., Zhu, Y., 2017. The International Heat Stress Genotype Experiment for modeling wheat response to heat: Field experiments and AgMIP-wheat multi-model simulations. Open Data J. Agric. Res. 3, 23–28.

Meehl, G.A., Tebaldi, C., 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305 (5686), 994–997.

Miyashita, K., Tanakamaru, S., Maitani, T., Kimura, K., 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ. Exp. Bot. 53 (2), 205–214.

Mohammadi, M., Karimizadeh, R.A., Naghavi, M.R., 2009. Selection of bread wheat genotypes against heat and drought tolerance based on chlorophyll content and stem reserves. J. Agric. Soc. Sci. 5, 119–122.

Monneveux, P., Rekika, D., Acevedo, E., Merah, O., 2006. Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. Plant Sci. 170 (4), 867–872.

Munné-Bosch, S., Alegre, L., 2004. Die and let live: leaf senescence contributes to plant survival under drought stress. Funct. Plant Biol. 31, 203–216.

Nicolas, M.E., Gleadow, R.M., Dalling, M.J., 1984. Effects of drought and high temperature on grain growth in wheat. Funct. Plant Biol. 11 (6), 553–566.

Niwas, R., Khichar, M.L., 2016. Managing impact of climatic vagaries on the productivity of wheat and mustard in India. Mausam 67 (1), 205–222.

Noohi, K., Fatahi, E., Kamali, G.H.A., 2009. Heat stress effects analysis on wheat crop in southern provinces. In: EGU2009-4441. 313 'Geophysical Research Abstracts' Vol. 11 European Geosciences Union.

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J., Villar, R., 2009. Causes and

consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182, 565–588.
Porter, J.R., Gawith, M., 1999. Temperatures and the growth and development of wheat: a review. Eur. J. Agron. 10 (1), 23–36.
Pradhan, G.P., Prasad, P.V., Fritz, A.K., Kirkham, M.B., Gill, B.S., 2012. Effects of drought and high temperature stress on synthetic hexaploid wheat. Funct. Plant Biol. 39 (3), 190–198.
Prasad, P.V.V., Staggenborg, S.A., Ristic, Z., 2008. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: Ahuja, L.R., Reddy, V.R., Saseendran, S.A., Yu, Q. (Eds.), Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes, Adv. Agric. Syst. Model. 1. ASA, CSSA, SSSA, Madison, WI, pp. 301–355.
Rötter, R.P., Appiah, M., Fichtler, E., Kersebaum, K.C., Trnka, M., Hoffmann, M.P., 2018. Linking modelling and experimentation to better capture crop impacts of agroclimatic extremes – a review. Field Crops Res (this issue).
Rane, J., Nagarajan, S., 2004. High temperature index for field evaluation of heat tolerance in wheat varieties. Agric. Syst. 79 (2), 243–255.
Reyer, C.P.O., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R.P., Bonfante, A., Lorenzi, F.D., Dury, M., Gloning, P., Jaoudé, R.A., Klein, T., Kuster, M.T., Martins, M., Niedrist, G., Riccardi, M., Wohlfahrt, G., Angelis, P.D., Dato, G.D., François, L., Menzel, A., Pereira, M., 2013. A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. Glob. Change Biol. 19 (1), 75–89.
Ristic, Z., Cass, D.D., 1992. Chloroplast structure after water and high-temperature stress in two lines of maize that differ in endogenous levels of abscisic acid. Int. J. Plant Sci. 153 (2), 186–196.
Ristic, Z., Bukovnik, U., Prasad, P.V., 2007. Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. Crop Sci. 47, 2067–2073.
Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., Mittler, R., 2004. When defense pathways collide: the response of *Arabidopsis* to a combination of drought and heat stress. Plant Physiol. 134, 1683–1696.
Salvucci, M.E., Crafts-Brandner, S.J., 2004. Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. Physiol. Plant. 120 (2), 179–186.
Shah, N.H., Paulsen, G.M., 2003. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant Soil 257 (1), 219–226.
Sharkey, T.D., 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. Plant Cell Environ. 28 (3), 269–277.
Silva, E.N., Ferreira-Silva, S.L., Fontenele, A.V., Ribeiro, R.V., Viégas, R.A., Silveira, J.A., 2010. Photosynthetic changes and protective mechanisms against oxidative damage subjected to isolated and combined drought and heat stresses in *Jatropha curcas* plants. J. Plant Physiol. 167, 1157–1164.
Skirycz, A., Inzé, D., 2010. More from less: plant growth under limited water. Curr. Opin. Biotechnol. 21 (2), 197–203.
Spiertz, J.H.J., 1974. Grain growth and distribution of dry matter in the wheat plant as influenced by temperature: light energy and ear size. Neth. J. Agric. Sci. 22, 207–220.
Stone, P., Nicolas, M., 1994. Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. Funct. Plant Biol. 21, 887–900.
Stone, P.J., Nicolas, M.E., 1995. A survey of the effect of high temperature during grain filling on yield and quality of 75 wheat cultivars. Aust. J. Agric. Res. 46, 475–492.
Tashiro, T., Wardlaw, I.F., 1990. The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat. Funct. Plant Biol. 17, 551–561.
Trnka, M., Olesen, J.E., Kersebaum, K.C., Skjelvåg, A.O., Eitzinger, J., Seguin, B., Peltonen-Sainio, P., Rötter, R., Iglesias, A., Orlandini, S., Dubrovský, M., Hlavinka, P., Balek, J., Eckersten, H., Cloppet, E., Calanca, P., Gobin, A., Vučetić, V., Nejedlik, P., Kumar, S., Lalic, B., Mestre, A., Rossi, F., Kozyra, J., Alexandrov, V., Semerádová, D., Žalud, Z., 2011. Agroclimatic conditions in Europe under climate change. Glob. Change Biol. 17 (7), 2298–2318.
Trnka, M., Brázdil, R., Olesen, J.E., Eitzinger, J., Zahradníček, P., Kocmánková, E., Dobrovolný, P., Štěpánek, P., Možný, Bartošová, L., Hlavinka, P., Semerádová, D., Valášek, H., Havlíček, M., Horáková, V., Fischer, M., Žalud, Z., 2012. Could the changes in regional crop yields be a pointer of climatic change? Agric. For. Meteorol. 166, 62–71.
Trnka, M., Rötter, R.P., Ruiz-Ramos, M., Kersebaum, K.C., Olesen, J.E., Žalud, Z., Semenov, M.A., 2014. Adverse weather conditions for European wheat production will become more frequent with climate change. Nat. Clim. Change 4, 637–643.
Trnka, M., Hlavinka, P., Semenov, M.A., 2015. Adaptation options for wheat in Europe will be limited by increased adverse weather events under climate change. J. R. Soc. Interface 12 (112), 20150721.
Trnka, M., Semerádová, D., Novotný, I., Dumbrovský, M., Drbal, K., Pavlík, F., Vopravil, J., Štěpánková, P., Vizina, A., Balek, J., Hlavinka, P., Bartošová, L., Žalud, Z., 2016a. Assessing the combined hazards of drought, soil erosion and local flooding on agricultural land: a Czech case study. In: Wilhite, A. D., Brázdil, R., Býntgen, U., Hayes, M., Trnka, M. (Eds.), Drought in Central Europe –from drought response to preparedness. Clim. Res. 70, 195–214.
Trnka, M., Olesen, J.E., Kersebaum, K.C., Rötter, R.P., Brázdil, R., Eitzinger, J., Jansen, S., Skjelvåg, A.O., Peltonen-Sainio, P., Hlavinka, P., Balek, J., Eckersten, H., Gobin, A., Vučetić, V., Dalla Marta, A., Orlandini, S., Alexandrov, V., Semerádová, D., Štěpánek, P., Svobodová, E., Rajdl, K., 2016b. Changing regional weather–crop yield relationships across Europe between 1901 and 2012. In: Wilhite, A. D., Brözdil, R., Bántgen, U., Hayes, M., Trnka, M. (Eds.), Drought in Central Europe – from drought response to preparedness. Clim. Res. 70, 195–214.
Tuba, Z., Lichtenthaler, H.K., Csintalan, Z., Nagy, Z., Szente, K., 1996. Loss of chlorophylls, cessation of photosynthetic CO_2 assimilation and respiration in the poikilochlorophyllous plant *Xerophyta scabrida* during desiccation. Physiol. Plant. 96, 383–388.
Ugarte, C., Calderini, D.F., Slafer, G.A., 2007. Grain weight and grain number responsiveness to pre-anthesis temperature in wheat barley and triticale. Field Crops Res. 100, 240–248.
Urban, O., Hlaváčová, M., Klem, K., Novotná, K., Rapantová, B., Smutná, P., Horáková, V., Hlavinka, P., Škarpa, P., Trnka, M., 2018. Combined effects of drought and high temperature on photosynthetic characteristics in four winter wheat genotypes. Field Crops Res (this issue).
Valério, I.P., Carvalho, F.I.F.D., Benin, G., Silveira, G.D., Silva, J.A.G.D., Nornberg, R., Hagemann, T., Sousa Luche, H.D., Oliveira, A.C.D., 2013. Seeding density in wheat: the more, the merrier? Sci. Agricola 70 (3), 176–184.
Vara Prasad, P.V., Bheemanahalli, R., Krishna Jagadish, S.V., 2017. Field crops and the fear of heat stress –opportunities: challenges and future directions. Field Crops Res. 200, 114–121.
Vassileva, V., Signarbieux, C., Anders, I., Feller, U., 2011. Genotypic variation in drought stress response and subsequent recovery of wheat (*Triticum aestivum* L.). J. Plant Res. 124 (1), 147–154.
Verma, V., Foulkes, M.J., Worland, A.J., Sylvester-Bradley, R., Caligari, P.D.S., Snape, J.W., 2004. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135, 255–263.
Wardlaw, I., Dawson, I., Munibi, P., Fewster, R., 1989. The tolerance of wheat to high temperatures during reproductive growth: I. Survey procedures and general response patterns. Aust. J. Agric. Res. 40, 1–13.
Wardlaw, I.F., 2002. Interaction between drought and chronic high temperature during kernel filling in wheat in a controlled environment. Ann. Bot. 90 (4), 469–476.
White, J.W., Webber, H., Kimball, B.A., Ewert, F., Asseng, S., Rezaei, E.E., Pinter, P.J., Hatfield, J.L., Reynolds, M.P., Ababaei, B., Bindi, M., Doltra, J., Ferrise, R., Kage, H., Kassie, B.T., Kersebaum, K.C., Luig, A., Olesen, J.E., Semenov, M., Stratonovitch, P., Ratjen, A.M., Martre, P., 2017. Modeling canopy temperature As a driver of heat stress in wheat. In: Paper Presented in Managing Global Resources for a Secure Future – 2017 Annual Meeting. Tampa Florida, USA, from 22 to 25 October, 2017.
Zheng, B., Chenu, K., Fernanda Dreccer, M., Chapman, S.C., 2012. Breeding for the future: what are the potential impacts of future frost and heat events on sowing and flowering time requirements for Australian bread wheat (*Triticum aestivum*) varieties? Glob. Change Biol. 18, 2899–2914.